Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of the claims in this application.

Listing of claims:

1. (Currently amended) A method for detecting a mutant polynucleotide in a mixture comprising mutant and wild-type polynucleotides ~~of mutant polynucleotides, wild-type polynucleotides and unrelated polynucleotides~~, comprising the steps of:

a) selecting an extension primer complementary to a first target sequence in both the mutant and wild-type polynucleotides;

b) selecting a probe complementary to a second target sequence in the wild-type polynucleotides but not in the mutant polynucleotides,

wherein the second target sequence in the wild-type polynucleotide is located 3' of the first target sequence on the same polynucleotide strand;

c) subsequently contacting the polynucleotides in the mixture with the probe under conditions where the probe preferentially anneals to the second target sequence on the wild-type polynucleotide strand ~~rather than to a corresponding sequence in~~ but does not anneal to the mutant polynucleotides;

d) subsequent to step (c), contacting the polynucleotides in the mixture with the extension primer under conditions where the primer anneals to the first target sequence; and

e) contacting the polynucleotides in the mixture with a DNA polymerase and nucleoside triphosphates under conditions where the extension primers are extended by polynucleotide synthesis, using the mutant and wild-type polynucleotides as templates, to produce extension products,

wherein, if the polynucleotides are present in the mixture in double stranded form as sense and antisense strands, only one of the sense or antisense strands for each of the wild type and mutant polynucleotides has a target sequence, such that its complimentary strand is not capable of being a template for extension, and

wherein polynucleotide synthesis that uses the wild-type polynucleotides as templates is blocked by the probe and polynucleotide synthesis that uses the mutant

polynucleotides as templates is not blocked by the probe and therefore produces a longer extension product.~~;~~

~~e) isolating the extension products from the mixture; and~~

f) detecting the presence of the mutant polynucleotide extension products. ~~amplifying the extension products produced from mutant polynucleotide templates preferentially over amplifying extension products produced from wild-type polynucleotide templates using the polymerase chain reaction (PCR).~~

2. (Original) The method of claim 1, wherein the mutant polynucleotides contain deletion mutations, insertion mutations, substitution mutations or a combination of deletion, insertion and substitution mutations, as compared to the wild-type polynucleotides.

3. (Currently amended) The method of claim 1, wherein the mutant and wild-type polynucleotides are isolated from the mixture of mutant polynucleotides, wild-type polynucleotides and unrelated polynucleotides before or after the step of contacting the polynucleotides with the probe.

4. (Currently amended) The method of claim ~~3~~ 28, wherein the mutant and wild-type polynucleotides are isolated using a sequence specific hybrid capture method.

5. (Original) The method of claim 1, wherein the extension primer has one or more attached biotin molecules.

6. (Original) The method of claim 1, wherein the probe is a peptide nucleic acid

7. (Original) The method of claim 1, wherein the probe is an oligonucleotide.

8. (Original) The method of claim 7, wherein at least part of the oligonucleotide has a phosphorothioated backbone.

9. (Original) The method of claim 7, wherein the oligonucleotide has a 5' end and a 3' end and the 3' end is modified such that it cannot be extended by polynucleotide synthesis.

10. (Original) The method of claim 9, wherein the nucleotide at the 3' end of the oligonucleotide is phosphorylated.

11. (Original) The method of claim 1, wherein the probe is a locked nucleic acid, modified oligonucleotide or oligonucleotide analogue.

12. (Original) The method of claim 1, wherein:

a) there is a first T_m for annealing of the extension primer to the first target sequence;

b) there is a second T_m for annealing of the probe to the second target sequence; and

c) there is a third T_m for annealing of the probe to the mutant polynucleotides,

wherein the second T_m is higher than the first T_m; and

wherein the first T_m is higher than the third T_m.

13. (Original) The method of claim 1, wherein the first target sequence and the second target sequence overlap.

14. (Canceled) ~~The method of claim 1, wherein polynucleotide synthesis preferentially extends extension primers that have annealed to the first target sequence in mutant polynucleotides.~~

15. (Original) The method of claim 1, wherein the extension products are isolated from the mixture by a solid phase extraction method.

16. (Original) The method of claim 1, wherein the extension products from mutant polynucleotides as templates are preferentially isolated from the mixture.

17. (Currently amended) The method of claim 1, further comprising the step of amplifying the extension products using PCR wherein the PCR uses;

a) a first PCR primer that is complementary to a nucleotide sequence present in the 3' end of a long extension product, but not present in a short extension product; and

b) a second PCR primer that is identical to a nucleotide sequence present in both the long and short extension products.

18. (Currently amended) The method of claim ~~1~~ 17, comprising the additional step of analyzing the amplified extension products from the PCR.

19. (Currently amended) A method for detecting a mutant microsatellite in a mixture of mutant microsatellites and wild-type microsatellites, in a sample of genome DNA from an individual, comprising:

a) first, contacting a probe with the microsatellites in the mixture under conditions where the probe preferentially anneals to a second target region in the wild-type microsatellites as compared to a corresponding region in the mutant microsatellites,

wherein the corresponding region in the mutant microsatellites differs in nucleotide sequence from the second target region in the wild-type microsatellites;

b) contacting an extension primer with the microsatellites in the mixture under conditions where the extension primer anneals to a first target region in both the mutant and wild-type microsatellites, that is on the same strand as and located 5' of the second target region in the wild-type satellites, and is on the same strand as and located 5' of the corresponding sequence in the mutant microsatellites~~;~~,

wherein, if the microsatellites are present in the mixture in double stranded form as sense and antisense strands, only one of the sense or antisense strands for each of the wild type and mutant microsatellites has a target sequence, such that its complimentary strand is not capable of being a template for extension;

c) contacting a polymerase and nucleoside triphosphates with the microsatellites in the mixture under conditions where polynucleotide synthesis extends the extension primers using the microsatellites as templates to produce extension products,

wherein polynucleotide synthesis that uses wild-type microsatellites as templates is preferentially blocked by the probe as compared to polynucleotide synthesis that uses mutant microsatellites as templates;

d) isolating the extension products from the mixture;

e) amplifying the extension products by PCR to produce PCR products,

wherein the extension products that used mutant microsatellites as templates are preferentially amplified as compared to extension products that used wild-type microsatellites as templates;

f) analyzing the extension products amplified by the PCR.

20. (Original) The method of claim 19, wherein the microsatellites are TGF-β RII (A)10 or BAT26 microsatellites.

21. (Original) The method of claim 19, wherein the microsatellites are NR-21 microsatellites.

22. (Original) The method of claim 19, wherein the genome DNA is from a stool or blood sample.

23. (Original) The method of claim 19, wherein a multiplexed assay is used to simultaneously detect two or more mutant microsatellites.

24. (Original) The method of claim 19 wherein a multiplexed assay is used to simultaneously detect mutant TGF-β RII (A)10 and BAT26 microsatellites.

25. (Currently amended) A method for selectively amplifying a mutant polynucleotide~~, if any,~~ in a mixture ~~of~~ comprising mutant and wild-type polynucleotides ~~and unrelated polynucleotides~~, both the mutant polynucleotide and the wild-type polynucleotides having the

same first target sequence, the wild-type polynucleotide having a second target sequence not present in the mutant polynucleotide, at least part of the second target sequence being located 3' of the first target sequence on the same polynucleotide strand of the wild-type polynucleotide, the process comprising:

a) first, contacting the mixture with a probe that is complementary to the second target sequence to preferentially anneal the probe to the second target sequence of the wild-type polynucleotide rather than to a corresponding sequence in the mutant polynucleotide;

wherein, if the polynucleotides are present in the mixture in double stranded form as sense and antisense strands, only one of the sense or antisense strands for each of the wild type and mutant polynucleotides has a target sequence, such that its complimentary strand is not capable of being a template for extension,

b) contacting the mixture with an extension primer complementary to the first target sequence in both the mutant and wild-type polynucleotides to anneal the primer to the first target sequence in both polynucleotides; and

c) contacting the mixture with a DNA polymerase and nucleoside triphosphates to extend the extension primers annealed to the polynucleotides by polynucleotide synthesis,

~~the probe annealed to the wild-type polynucleotide limiting polynucleotide synthesis of the extension primer annealed to the wild-type polynucleotide~~

wherein polynucleotide synthesis that uses the wild-type polynucleotides as templates is blocked by the probe and polynucleotide synthesis that uses the mutant polynucleotides as templates is not blocked by the probe and therefore produces a longer extension product.

26. (Original) The process of claim 25, further comprising:

a) isolating extended extension primers from the mixture; and

b) contacting the extended extension primers with a first and second PCR primer, a PCR polymerase and nucleoside triphosphates to amplify the extended extension primers by PCR,

the PCR preferentially amplifying extended extension primers from extension primers annealed to mutant polynucleotides.

27. (Currently Amended) The process of claim ~~25~~ 26, further comprising determining the size and abundance of amplified extended extension primers from the PCR.

28. (New) The method of claim 3, further comprising the step of isolating the extension products from the mixture.

29. (New) The method of claim 28, further comprising the step of amplifying the extension products produced from mutant polynucleotide templates.

30. (New) The method of claim 1, further comprising the step of amplifying the extension products produced from mutant polynucleotide templates.